nating to explore and nothing less than extraordinary. A considerable number of challenges remain to establish a connection between the current data, derived from experiments in yeast and *Aplysia*, and mammalian neurons. Intricacies abound; for example, the recent finding that CPEB may be involved in localizing CPE-containing mRNAs to the dendrite (Huang et al., 2003), raising the point that prion-like switches, if they do occur in mammalian neurons, could affect various aspects of protein biology. One further suspects that at least in mammals, neuronal RNA binding proteins in addition to CPEB may also play roles in the regulation of synaptic RNAs. For instance, the fragile-X mental retardation syndrome results from the lack of an RNA binding protein believed to be present in the synapse and to play a role in synaptic plasticity (Jin and Warren, 2003). Added to the issue of additional RNA binding proteins is the possibility that at least some neuronal RNA binding proteins, including FMRP and components of the survival of motor neuron protein complex, may regulate both neuronal mRNAs and miRNAs (Dostie et al., 2003; Jin and Warren, 2003). Identification of key RNA binding proteins involved in synaptic plasticity thus whets one's appetite for knowing what RNAs are being regulated. This question seems critical for developing a more complete understanding of the molecular nature of synaptic plasticity and has begun to be addressed by the development of new methodologies (Ule et al., 2003 and references therein).

Relating the current work to mammalian neurons will require establishing a clearer relationship between the ApCPEB and mammalian isoforms of CPEB. One CPEB transcript appears to be brain specific in mice (mCPEB-3), differing from other isoforms in that it harbors an extra 2500 bp of 3′ UTR; this transcript encodes an N terminus with a small Q-rich domain—50% in the first 30 amino acids, but then an unimpressive extension in which the following 130 amino acids have only 10% Q + N, an average content (Theis et al., 2003). Other mCPEB variants do not clarify this issue. If mammalian CPEB proteins do prove capable of forming prion-like switches on the basis of such short Q-rich domains, one wonders how many other proteins might show similar phenomenon. For example, a number of proteins with Q-rich N termini are implicated in neurologic disorders (e.g., Huntington's, spinocerebellar ataxia). If the phenomenon were widespread, one could imagine great expansion of the current CPEB-dependent switching model for generating synaptic marks. More generally, perhaps the findings reviewed here will pave the way to demonstrating, as suggested by Lindquist, that prion-like switches play a number of important roles in mammalian cells.

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The Enemy at the Gates: Ca^{2+} Entry through TRPM7 Channels and Anoxic Neuronal Death

In brain ischemia, gating of postsynaptic glutamate receptors is thought to initiate Ca^{2+} overload leading to excitotoxic neuronal death. In this issue, Aarts and colleagues describe a novel mechanism, whereby gating of TRPM7, a Ca^{2+}-permeable nonselective cation channel, mediates Ca^{2+} overload and demise of anoxic neurons.

The term excitotoxicity describes the pathological consequences of the overstimulation of glutamate receptors (Olney, 1969), which occurs in brain ischemia/anoxia, epilepsy, and brain trauma. Excitotoxic events may also be involved in several neurodegenerative diseases including HIV dementia and Alzheimer's and Huntington's disease (Beal, 1992; Lipton, 1996). Excitotoxicity is due in large part to overstimulation of the N-methyl-D-aspartate receptor (NMDA-R), with sustained gating of its associated ion channel, which allows inflow of massive amounts of Ca^{2+} and Na^{+} in neurons. Ca^{2+} overload can trigger several downstream lethal reactions, including nitrosative and oxidative stress, mitochondrial dysfunction, and protease and phospholipase activation, which culminate in cell death (Orrenius et al., 2003). It has been recognized that lethal signals in brain ischemia are not exclusively mediated through the NMDA-R associated channel. For example, both glutamate metabotropic receptors and voltage-regulated L-type channels have been implicated in the mechanisms leading to cell death in anoxic/ischemic brain.

In this issue of *Cell*, Aarts and colleagues (2003) use a model of hypoxia (oxygen/glucose deprivation; OGD) to unveil a new lethal pathway that involves the activation of a cation conductance (I_{OGD}), which results in Ca^{2+} overload. I_{OGD} requires the TRPM7 ion channel protein, a member of the TRP (transient receptor potential) cation

channel superfamily (Harteneck et al., 2000). In OGD, reactive oxygen/nitrogen species gate I_{OGD}/TRPM7, which allows Ca^{2+} entry into the neuron. This triggers a vicious loop since Ca^{2+} overload stimulates further radical production and I_{OGD}/TRPM7 activation. Significantly, this pathway is unmasked by inhibiting the classical Ca^{2+} entry routes through the NMDA-R and the L-type channels. Depletion of TRPM7 by sRNAi protects neurons from Ca^{2+} overload and the consequences of prolonged anoxia. Surprisingly, TRPM7 seems to be essential in mediating Ca^{2+} overload and cell death, even in the absence of the NMDA and L-type channel block, which suggests a central role for this pathway in both excitotoxic and nonexcitoxic neuronal demise. The authors suggest that these findings can explain the failure of conventional antiexcitotoxic therapy (AET), which has been largely based on the use of NMDA antagonists. Conventional AET would only block excitotoxicity, but eventually unmask the predominant pathway for Ca^{2+} overload, via TRPM7.

Aarts and colleagues (2003) provide evidence that I_{OGD} involves TRPM7 and that activation of TRPM7 is mediated by ROS. Both excitotoxocity and anoxia can promote radical formation, which would gate TRPM7 and promote Ca^{2+} overload and radical production. However, the mechanism whereby ROS can activate TRPM7 during anoxia remains unclear. Also, high intracellular Mg^{2+} levels may negatively regulate the channel during ischemia.

Whereas the novel redox regulation of TRPM7 is very intriguing, perhaps of wider impact is the finding that suppression of TRPM7 prevents neuronal demise both in the presence and in the absence of NMDA blockade. This implies that the new pathway is required also for excitotoxicity. How does this novel pathway change our current knowledge of brain ischemia and excitotoxicity? The authors suggest that anoxia would trigger both excitotoxicity and I_{OGD}. However, anoxia rapidly suppresses synaptic activity and although glutamate is released by both synaptic and nonsynaptic mechanisms, NMDA-R is quickly desensitized. Increasing the duration of anoxia, excitotoxicity would become less important, whereas gating of TRPM7 would become the most relevant route to promote Ca^{2+} accumulation. The effect of TRPM7 suppression in the absence of NMDA channel blockers suggests that I_{OGD} may also be relevant in the early phases of anoxia to enhance the loop between Ca^{2+} overload and radical production. In this respect, I_{OGD} would not be dissimilar from other receptors/channels, which have been shown to participate in excitotoxicity. The authors suggest that the new pathway may be more relevant than excitotoxicy in determining the lethal consequences of anoxia in brain. Nevertheless, experiments in ischemic animal models are necessary to decide which of the two lethal pathways is dominant. It is plausible that the excitotoxic and the novel nonexcitotoxic pathway synergize to promote Ca^{2+} overload and radical production in a feed-forward loop. Because TRPM7 is expressed in nonexcitable tissues, it is tempting to speculate that it may have a widespread role in mediating anoxic injury also in tissues such as liver and lung. However, if TRPM7 were the predominant lethal effector of anoxia, it would be difficult to explain the high sensitivity to anoxia of brain and heart, when compared to liver or lung, given that cells are equally intolerant to prolonged Ca^{2+} overload.

The discovery of a new gate leading to neuronal death opens an exciting new spectrum of possibilities for therapy of brain hypoxia and potentially other neuropathological conditions. While the authors suggest that a fundamental reason for the apparent failure of AET in clinical trials is the unmasking of the newly described pathway, they recognize that the reasons may be several and more complex. Considerable effort has been put into developing NMDA antagonists to treat brain ischemia and other disorders where excitotoxicity may be involved. However, NMDA activity is essential for normal neuronal function. Modern drugs are generally designed to have high affinity and selectivity for their target, especially those generated generally by high-throughput screening. High-affinity NMDA blockers (i.e., MK-801) have unacceptable side effects, which largely explain the failure of clinical trials. In contrast, more recent approaches that use noncompetitive NMDA antagonists, with a fast offrate from the channel (Lipton and Chen, 2004), are more successful and have no major side effects. Neuronal demise and neurological dysfunction in brain ischemia are clearly due to several components. Different therapeutic strategies including thrombolysis, AET, and eventually drugs acting on TRPM channels may have to be combined to achieve success.

Excitotoxic neuronal loss is not due to one mechanism, but to overlapping death routines, including necrosis, apoptosis (Ankarcrona et al., 1995), and, possibly, autophagic cell death (Yue et al., 2002). Aarts and colleagues show that death due to prolonged anoxia is of a nonapoptotic type. This is not surprising because significant amounts of ATP are required to activate the apoptosome. Even when ATP/ADP ratio is retained, the bulk requirement for ATP may be insufficient for apoptosis (Leist et al., 1997). Apoptosis contributes to ischemic brain damage and brain infarction can be reduced by treatment with caspase inhibitors, which may block both the proinflammatory response and the execution of a subset of cell death.

The last very intriguing aspect of the findings presented by Aarts and colleagues is that different death routines can be recruited at different times, and each may become more or less relevant as the injury progresses. Further work is required to determine the relative contribution of ion-dependent and -independent death pathways. The identification of a new important route that promotes cell disruption will help in designing new strategies to keep the enemy from the gates.

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